

pH and Solvent Viscosity Effects of D-Arginine Dehydrogenase with Different D-Amino Acid Substrates

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D-Arginine dehydrogenase (DADH) is a flavin-dependent enzyme that requires FAD as a cofactor for catalysis (1). It is found in *Pseudomonas aeruginosa*, a common opportunistic human pathogen (2). DADH catalyzes the oxidation of D-arginine to iminoarginine, which is subsequently converted to ketoarginine and ammonia in solution (1). Previous studies have established that the enzyme is active on all the D-amino acids, with the exception of D-glutamate, D-aspartate, and glycine (3). Based on the k_{cat}/K_m values at pH 8.7, the best substrates for the enzyme are D-arginine (i.e., $10^6 \text{ M}^{-1}\text{s}^{-1}$) followed by D-lysine, D-tyrosine, D-methionine, D-phenylalanine, D-histidine, and D-leucine (3).

In this study, the pH profiles of the steady state kinetic parameters with D-histidine and D-methionine as the substrate for the enzyme were determined in order to establish the contribution of the ionizable side chain of the substrate to catalysis. These two amino acids were chosen based on their comparable k_{cat}/K_m values at pH 8.7 (i.e., 10^3 - $10^4 \text{ M}^{-1}\text{s}^{-1}$) (3). The resulting pH profiles showed differences in the number of ionizable groups participating in enzyme turnover, suggesting the importance of the correct ionization state of the side chain of the substrate for optimal enzyme activity.

Solvent viscosity effects were studied in the pH independent region at pH 9.7 to investigate whether diffusion-controlled events influence the binding of the substrate and the release of the product to and from the enzyme. The solvent viscosity studies were investigated with not only D-methionine and D-histidine, but also D-arginine and D-lysine, which are the preferred substrates by DADH. Steady state kinetic parameters and solvent viscosity effects will be closely examined alongside available x-ray crystallography structures to unveil the differences in enzyme turnover among the various substrates.

REFERENCES

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